# Nuclear Alterations during Spermiogenesis of *Triatoma infestans* (Hemiptera, Reduviidae)

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ABSTRACT

The pattern of spermiogenesis in insects as described for some orders has been generally accepted as the process typical for this taxon. However some species depart from this pattern at specific steps and these differences may be used as taxonomic characteristics. In *Triatoma infestans*, the most striking difference is the lack of nuclear grooves and adjacent membranes which are substituted by coated nuclear protrusions occurring during nuclear elongation. Ten stages of spermiogenesis are recognized and described.

RÉSUMÉ

**Altérations nucléaires pendant la spermiogenèse de *Triatoma infestans* (Hemiptera, Reduviidae)**

Le processus de spermiogenèse des insectes, tel qu'il a été décrit pour certains ordres, a été généralement accepté comme le processus typique pour ce taxon. Toutefois, certaines espèces se démarquent de ce processus dans des étapes spécifiques et ces différences peuvent être utilisées comme des caractéristiques taxonomiques. Chez *Triatoma infestans*, la différence la plus marquante est l'absence de gouttières nucléaires et de membranes adjacentes qui sont remplacées par des protubérances nucléaires revêtues apparaissant pendant l'élongation nucléaire. Dix stades de spermiogenèse sont reconnus et décrits.

The differentiation of spermatids into spermatozoa is a profound transformation in which all organelles are greatly modified in structure and function, or eliminated when the cell has no further necessity for their contribution towards maturation. To define this process in an orderly manner, it was divided into specific stages by various authors. First described [15] in *Drosophila melanogaster*, the same steps were found in other dipterans such as *Ceratitis capitata* [13] and *Chrysomya megacephala* [11]. Only small differences were noted in other orders such as Orthoptera [16], and Coleoptera [10].

Some isolated aspects of hemipteran spermiogenesis were included in review articles [2, 3]. Aspects of this differentiation process have been thoroughly investigated, such as the formation of the Nebenkern or mitochondrial complex [12]. The nature of the mitochondrial derivatives filled with a paracrystalline structure was investigated in various insects including Hemiptera [4, 14].

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The flagellar characteristics of various hemipteran spermatozoa have also been investigated [1, 6-8]. These studies show a strong resemblance between the flagella of the spermatozoa of various genera [7] and even of different families [1].

This is not the case in nuclear and acrosome development. A completely distinct nuclear and acrosome organization was described for the spermatozoa of *Saldula saltatoria* and *Chartoscirta cineta*, where the acrosome is sucker-shaped and the nucleus extends into the flagellum [1]. The development of *Cerris puludum* is another variation in hemipteran spermiogenesis in which complex acrosome and nuclear forms were found in late spermatids [19]. Also in the giant spermatozoon of *Notonecta glauca*, the development of its very long acrosome and nucleus is very different from the pattern known for other Heteroptera [18]. A report on the last stages of spermiogenesis of *Leptocoris trivittatus* (Hemiptera, Corizidae) is more similar to the hemipteran investigated in this study, although acrosome location in a nuclear canal is also distinct from other known species [9]. The most complete description of the nuclear region of an hemipteran during spermiogenesis can be found in relation to *Carabus catenulatus* and *Nepa rubra* [17]. Unfortunately the difference in the methodology used makes some structures difficult to compare.

## MATERIAL AND METHODS

Final (5th) stage male nymphs of *Triatoma infestans* were prepared according to routine methods of electron microscopy for determining ultrastructure, using glutaraldehyde and osmium tetroxide. In the case of the specimens seen in figures 2 and 7, the post-fixation in osmium tetroxide was omitted.

## RESULTS

The early stages of spermatid development have spherical nuclei, similar to somatic cell nuclei, with finely granular chromatin which condenses only in contact with the nuclear membrane. The subdivision into stages is therefore based on cytoplasmic features, such as the Nebenkern formation in stage 2 (Fig. 1), its separation into two mitochondrial derivatives on each side of the axoneme (stage 3) and their elongation together with the axoneme (stage 4).

The last spherical nucleus stage (5th) is marked by the fusion of the proacrosome vesicle onto the nuclear membrane and its transport to the anterior region of the nucleus. Up to this stage, nucleoli can still be seen, suggesting an active cellular metabolism, necessary for the intense cytoplasmic modifications occurring at this time. Nuclear pores are limited to the region surrounding the basal plaque in which the centriole is anchored (Fig. 2). However, nuclear modification has already begun, as indicated by the first condensations of chromatin into thicker strands (Fig. 3).

In cross sections, the elongating nucleus of stage 6 has two pronounced projections covered by layered dense material (Fig. 4). Chromatin is more densely packed in these

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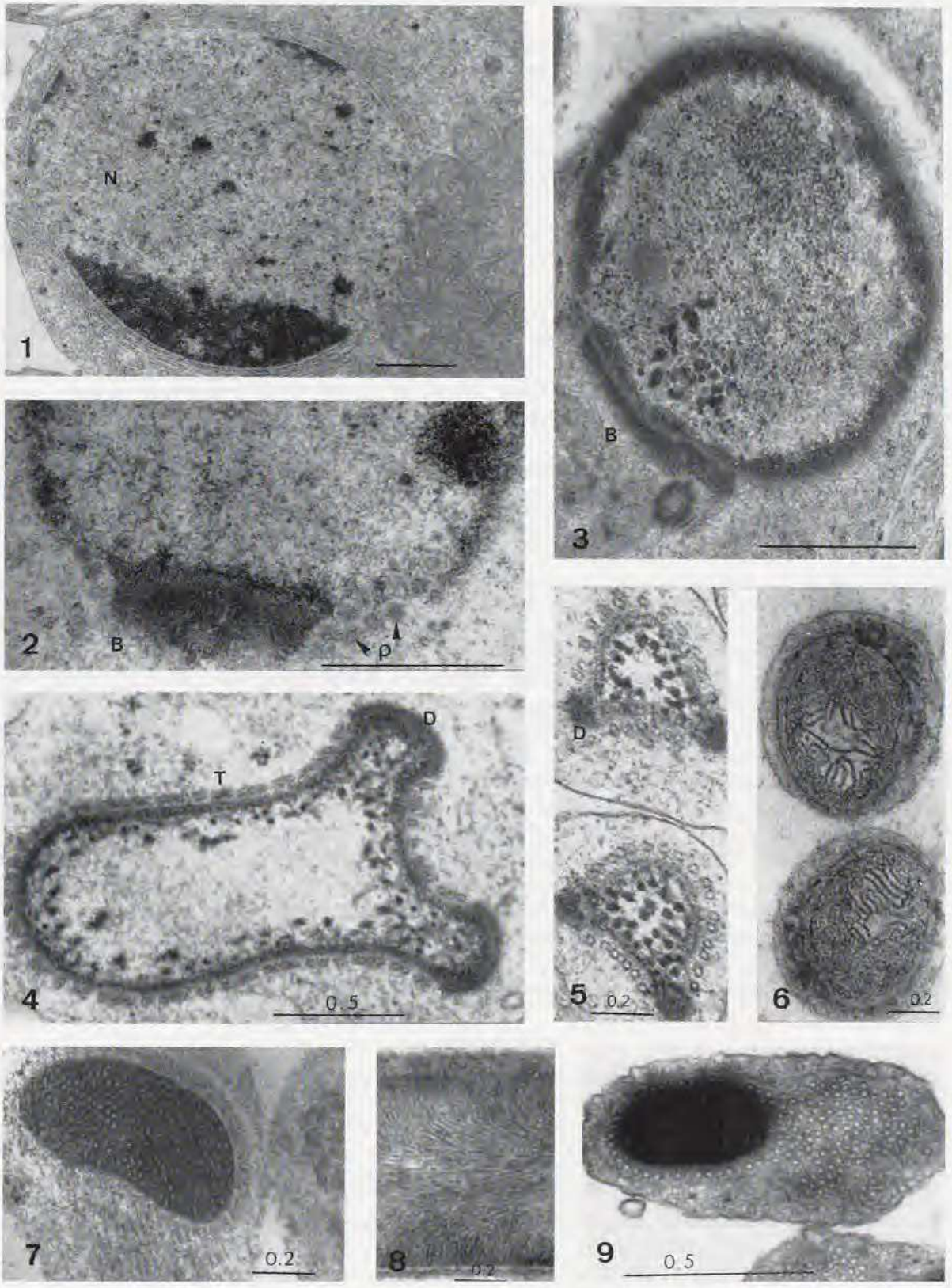
FIGS. 1-9 — Spermiogenesis in *Triatoma infestans*. **1:** Stage 2 spermatid has a round nucleus (N) in which the chromatin begins to condense near the nuclear membrane. A large mitochondrial complex or "Nebenkern" forms at the nuclear base. Bar = 1 µm. **2:** In stage 5, nuclear pores (P) are limited to the periphery of the basal plate (B). **3:** A stage 5 nucleus begins to form chromatin cords of various thicknesses. The basal plate (B) of dense granular material marks the centriolar insertion. **4:** Nuclear elongation and formation of thick chromatic cords identify stage 6. The transversely sectioned nucleus is surrounded by parallel microtubules (T) with the exception of two nuclear projections coated with a dense layer (D). **5:** In stage 7, elimination of the nuclear matrix diminishes the nuclear diameter and the nuclear projections. The dense coating is now limited to small tufts. **6:** Fusion of cords into chromatin lamellae marks stage 8. **7:** A rearrangement of the lamellae to form a regular network indicates stage 9. **8:** A longitudinal section in stage 9 also shows the regular, parallel, chromatin arrangement. **9:** In the last stage (stage 10) of spermatid development, the nucleus is very dense and no substructure of the chromatin can be seen. Cytoplasmic microtubules will be discarded with the excess cytoplasm in the spermatozoon.



protrusions than along the rest of the nuclear membrane which is enclosed externally by a row of parallel microtubules.

In stage 7, elongation is completed and the chromatin has been completely condensed into thick cords (Fig. 5). The nuclear projections are progressively reduced and the dense material which covered them is disorganized into tufts and disintegrates in the cytoplasm.

Stage 8 is identified by lamellar organization of the chromatin (Fig. 6), which rapidly branches out into a close network which identifies stage 9 (Figs. 7 and 8). A longitudinal section shows the compact parallel arrangement of the chromatin lamellae (Fig. 8).

Continued condensation results in a very dense nucleus in which the chromatin organization can no longer be visualized (Fig. 9). Only the sloughing off of the extra cytoplasm and microtubules separate this stage 10 spermatid from the mature spermatozoal structure.

## DISCUSSION

Despite the many studies on hemipteran spermatogenesis, these generally concentrate on an interesting aspect of their development and have not detailed the whole process. However, the step by step examination of this process shows that the same subdivision into stages can be applied to this group as has been described for a dipteran [15], an orthopteran [16] and a coleopteran [10].

The active nucleus is practically unmodified, accompanied by major reorganization of the cytoplasmic organelles, during the first stages of spermiogenesis. As nuclear elongation and chromatin condensation begins, these organelles are formed and are gradually modified as maturation proceeds. The next subdivisions of this process are, therefore, based on the nuclear alterations.

Only in stages six and seven are there pronounced differences in relation to the other insects studied. In these previous studies the nucleus forms deep nuclear grooves which are externally lined by the "adjacent membranes", a dense layer of the same thickness as a membrane, which later separates from the nucleus and curls in the cytoplasm where it remains until it is discarded together with the excess cytoplasm and microtubules.

In *Triatoma infestans*, the nuclear protrusions can be described as a new feature, apparently homologous in function with the nuclear grooves. The dense covering of these protrusions is very different from the adjacent membranes, not only in structure but also in its progressive disappearance at the end of stage 7 when the nuclear protrusions are absorbed and the dense material breaks up, becoming indistinguishable in the cytoplasm.

The greater condensation of the chromatin in the protrusions seems to indicate a role in chromatin condensation into thick cords and their organization along the length of the nucleus.

Stages 8 and 9 were rarely encountered and this has been interpreted as an indication that the events of chromatin lamination, network formation and condensation into a very dense, uniform nucleus occur very rapidly. Considering this fact, it may be more appropriate to unite the eighth and ninth stages as a single, more frequently encountered phase of spermiogenesis.

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